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Synthesis and Basicity of 2,6-Di-[2-(heteroaryl)vinyl]pyridines (°)

Alberto Ballistreri, [a] **Luisa Gregoli,** [a] **Giuseppe Musumarra,** *[a] **and Anna Spalletti** *[b]

[a] *Dipartimento di Scienze Chimiche, Università di Catania, Viale Andrea Doria 6, 95125 Catania, Italy.*
[b] *Dipartimento di Chimica, Università di Perugia, Via Elce di Sotto 8, 06123 Perugia, Italy.*

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Abstract: The synthesis and spectroscopic characterization of 2,6-di-[2-(heteroaryl)vinyl]pyridines (heteroaryl = 2-furyl, 2-thienyl, 2-pyridyl, 2-thiazolyl) is reported. The pKa of the above pyridine derivatives measured spectrophotometrically are coincident with those measured spectrofluorimetrically, indicating no acid-base re-equilibration in the excited state. NMR spectroscopy provides evidence for the central pyridine ring nitrogen as the site of first protonation in the tri-pyridine derivative.

INTRODUCTION

Compounds able to interact directly with nucleic acids are of great importance in cancer chemotherapy. In particular, drugs able to exert their biological effects by binding reversibly to double-helical B DNA either by intercalation or by noncovalent binding to the AT-rich regions of the minor groove of DNA, such as Netropsin, Distamycin, Berenil, Pentamidine [1-3], include aromatic or heteroaromatic moieties linked by carbon and/or heteroatom linkers, and amidino groups attached to the aromatic rings. Recently, replacement of the central linear linkers in pentamidine and berenil with a furan moiety provided more active compounds with superior DNA-binding properties, as confirmed by the crystal structures of drug-DNA complexes [4].There is a great deal of interest in the search for new structures able to bind selectively DNA minor groove GC bases [5,6] in which several oncogens are particularly rich.The fact that the linker, which may not be directly involved in the DNA binding, is decisive for the binding selectivity, suggested the design of structural skeletons in which heteroaromatic rings which may act as binders are linked by ethylenic double bonds exerting only a "spacing" function, as previously suggested for a vinyl-3,5-pyridine polymer [7].

In this context we here report on the synthesis and spectral features of 2,6-di-[2-(heteroaryl)vinyl]pyridines **5**, with the heteroaromatic rings in *trans* with respect to the ethylenic double bond. For a better characterization of the above compounds in view of their possible biological applications, we also report the pKa of the heteroaromatic nitrogen atoms and a NMR study to determine the site of first protonation in the terpyridine derivative **5c**.

(°) Dedicated to Professor A. R. Katritzky on the occasion of his 70th birthday.

(*) To whom correspondence should be addressed.

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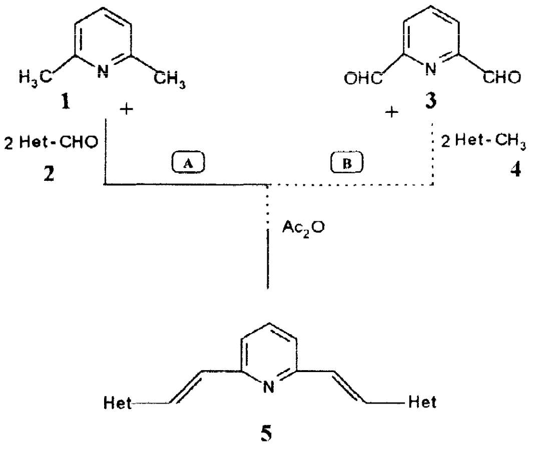
RESULTS AND DISCUSSION

The synthesis of 2,6-di-[2-(heteroaryl)vinyl]pyridines **5** (Scheme 1), was achieved by condensation of heteroaromatic aldehydes **2** with 2,6 lutidine **1** (Method A) in acetic anydride exploiting the acidic character of methyls adjacent to the pyridine ring nitrogen. The tripyridine derivative **5c** was prepared by condensation of 2,6 pyridindicarboxaldehyde **3** with 2-picoline **4** (Method B). All divinyl pyridines **5**, characterized by IR, NMR and mass spectrometry, are in *trans-trans* configuration as confirmed by the J coupling constants in the ^{1}H NMR spectra.

Scheme 1



	Het
(a)	furan-2-yl
(b)	thiophen-2-yl
(c)	pyridin-2-yl
(d)	thiazol-2-yl
(e)	phenyl

Basicity of 2,6-di-[2-(heteroaryl)vinyl]pyridines 5

Pyridine derivatives **5** were characterized spectrophotometrically in non-polar solvents. Their UV spectra are rather complicated and reveal the presence of two or three not well separated electronic transitions. A possible contribution to the shape of the spectra could also be due to the presence of *s-cis* - *s-trans* conformers, as observed in the corresponding distyryl-derivatives of benzene and naphthalene [8]. The first absorption band shows a maximum in the region 340-360 nm for all five compounds under study. Polar solvents (MeCN or buffered H_2O/MeCN mixtures) at neutral or basic pH do not change substantially the shape of the spectra but reduce their fine structure. In acidic solution, the intensity of the short wavelength bands decreases, while appearance of a new intense band in the range 350-400 nm (Fig. 1) allows spectrophotometric titrations to be easily carried out. Figure 2 shows as an example the spectral changes as a function of pH for PT_2.

A parallel fluorimetric investigation was performed in aqueous solutions. All these compounds exhibit in basic solutions emission maxima with good efficiency in the range 390-450 nm and below pH 2, with a strongly reduced intensity in the spectral range 420-520 nm, (see Fig. 1). Therefore, a fluorimetric titration is also possible, as shown in Figure 3 for PF_2 and in Figure 4 for P_3.

Table 1 collects the dissociation constants of the conjugate acids of the compounds investigated measured in MeCN/H_2O (15/85, v/v).

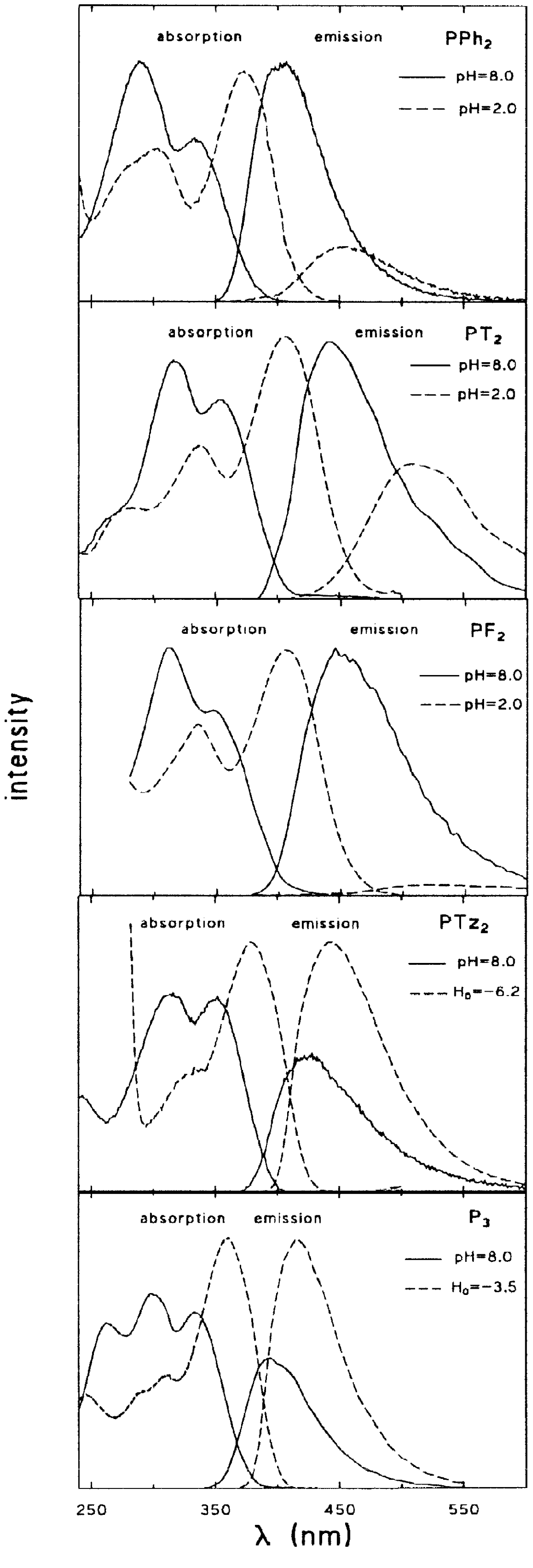


Fig. 1 - Absorption and fluorescence spectra of the four 2,6-di-[2-(heteroaryl)vinyl]pyridines and 2,6-distirylpyridine in H_2O/MeCN at different pH: ——neutral form, - - - - fully protonated form (H_0: acidity function, from M.A. Paul and F. A. Long, *Chem. Rev.*, 1957, 57, 1).

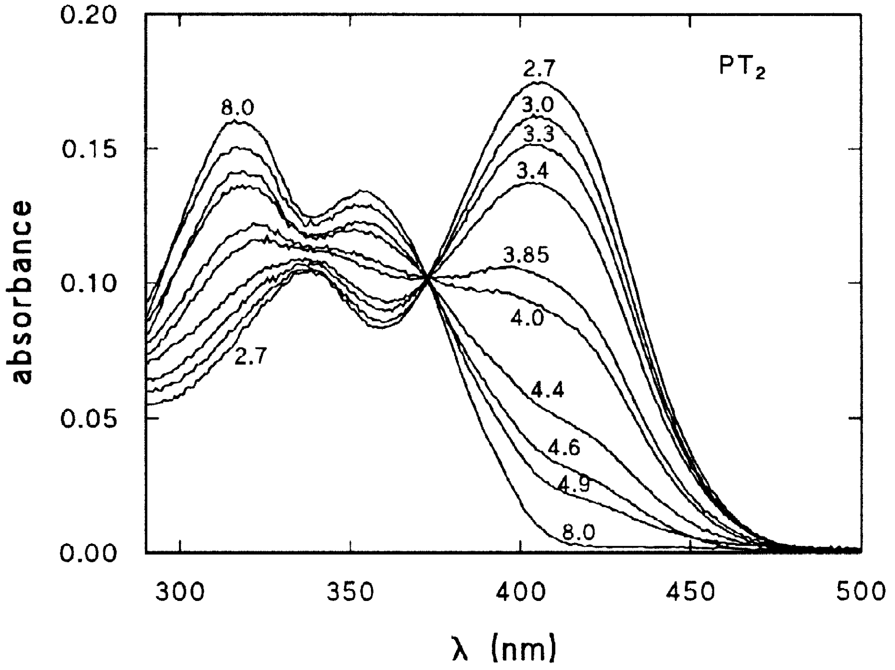


Fig.2 - Absorption spectra of PT_2 in H_2O/MeCN (85/15, v/v) as a function of pH.

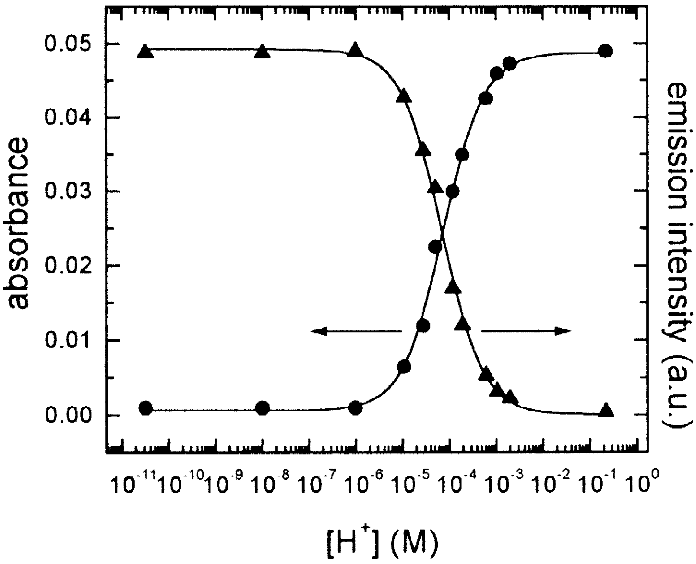


Fig. 3 - Fluorimetric titration curve at 438 nm (triangles) of PF_2 compared with the spectrophotometric titration curve at 420 nm (circles).

Table 1- pK_a values for 2,6-di-[2-(heteroaryl)vinyl]pyridines **5a-5d** and of 2,6-distyrylpyridine **5e** determined by different methods in $MeCN/H_2O$ (15/85, v/v)

compound	code[a]	pK_a (spectroph.)	pK_a (fluorim.)
5a	PF_2	4.2	4.2
5b	PT_2	4.0	4.3
5c	P_3	4.6, 1.0	4.9, 0.9, -1.1
5d	PTz_2	2.8, 1.1, (-3.1)	3.1, 0.9, (-5.5)
5e	PPh_2	4.3	4.3

[a] Indicates the central and the terminal aromatic rings: Ph= phenyl, P=2-pyridyl, F=2-furyl, T=2-thienyl, Tz=2-thiazolyl.

The absorption and emission spectra of the compounds with more than one protonation center (P_3 and PTz_2) change below pH 2. The emission spectrum changes markedly in intensity allowing measurement of the second and, in principle, of the third protonation in the region of negative pHs. Fig. 1 shows the spectra tentatively assigned to the fully-protonated forms. Table 1 and figure 4 provide evidence for the complementarity of these two spectroscopic methods in determining the pKa. However, the high ionic strength in concentrated $HClO_4$ solutions (up to $H_0 \sim -6$) can affect the spectral behaviour, thus increasing the uncertainty of of the experimental measurements.

The agreement in the pK_a values for the first and second protonation obtained by both methods indicates that no acid-base equilibration takes place in this pH range during the short excited state lifetime. As to the third protonation of P_3, useful information was obtained from the fluorescence spectra only, since the absorption spectra of $P_3H_2^{++}$ and $P_3H_3^{+++}$ species appear to be quite similar. Considering the good fit of the fluorimetric titration (see Fig. 4) we believe that the pK_a value of the third protonation in Table 1 is reliable enough. On the other hand, both titration methods showed a third inflection for PTz_2 in very acidic pHs. However, these two inflections occur at rather different pHs (see values in parentheses in Table 1). At present, there is not a reasonable explanation for this behaviour, which calls for further investigation.

The pK_a values for the first protonation of the reference compound distyrylpyridine **5e** is one pKa unit lower with respect to pyridine, the two styryl groups in ortho position appear then to behave as electron acceptors, in agreement with available σ^* values for C_6H_5-CH=CH: 0.41 [9] and 0.51 [10]. The expected stabilization of the protonated species due to the +M effect is then overbalanced by the -I effect as well as by some steric hindrance. The heteroaryl analogues PF_2, PT_2 and P_3 exhibit quite similar basicity while the PTz_2 derivative shows a rather lower pK_a of ~ 3 indicating a strong -I effect of the thiazole moieties. This heteroaryl group, whose ring N exhibits a pK of 2.52 [11], decreases the basicity of the first protonation site in the central ring by more than one pK unit. Obviously, further protonations at the N atom of the side rings occur at lower pH as compared to P_3.

In the 2-thiazolyl derivative **5d** the site of first protonation can be reasonably assumed to be the pyridine nitrogen atom, due to the above mentioned [12] difference in the basicity of pyridine (pKa = 5.2) with respect to thiazole (pK_a = 2.5). For the ter-pyridine derivative **5c**, in order to establish which pyridine ring nitrogen undergoes protonation first, supplementary evidence from NMR spectroscopy is needed.

NMR evidence for the site of first protonation in 5c

The ^1H-NMR spectrum of **5c** at pH=3 in CD_3CN/D_2O (15/85, v/v) and D_2SO_4, reported in figure 5, shows 8 signals whose integration and splitting pattern allows the following attribution:

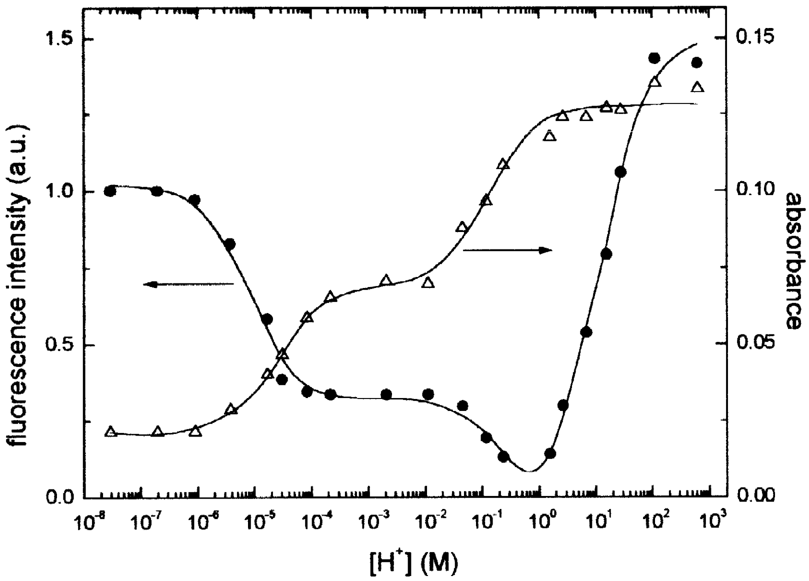


Fig. 4 - Fluorescence titration curve at 422 nm (circles) of P_3 compared with the spectrophotometric titration curve at 374 nm (triangles)

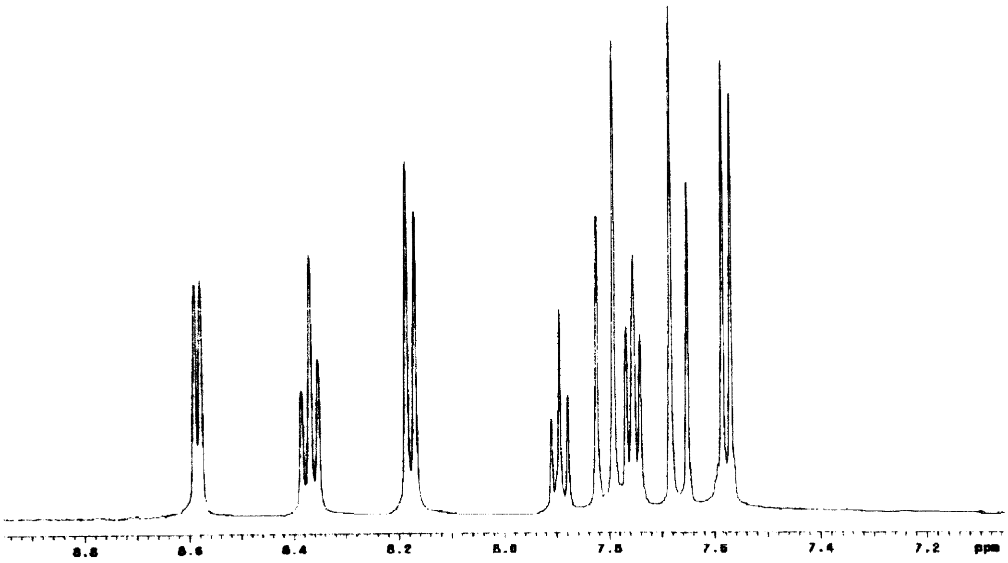
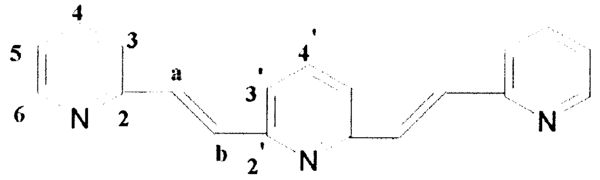


Fig. 5 - ^1H NMR spectrum of **5c** at pH=3 in CD_3CN/D_2O (15/85, v/v) and D_2SO_4

δ = 8.584 ppm (d, 2H, J=5.5 Hz) H_6
δ = 8.370 ppm (t, 2H, J=8Hz) H_4
δ = 8.178 ppm (d, 2H, J=8Hz) H_3
δ = 7.895 ppm (t, 1H, J=8Hz) H_4'
δ = 7.808 ppm (d, 2H, J=16Hz) H_a or H_b
δ = 7.754 ppm (t, 2H, J=6.5 Hz) H_5
δ = 7.668 ppm (d, 2H, J=16Hz) H_a or H_b
δ = 7.577 ppm (d, 2H, J=7.5 Hz) H_3'



The ethylenic protons coupling constants (15-16 Hz) are characteristic for those in *trans* with respect to the double bond, indicating that the molecule is in the *trans-trans* configuration. The aforecited 8 signals, each integrating for two protons except the $H_{4'}$ triplet which integrates for one proton only, clearly account for the simmetry of the molecule. In fact, in case of protonation at the central pyridine ring nitrogen, the molecule should retain its simmetry and exhibit a simplified spectrum with 8 signals corresponding to 8 magnetically equivalent protons, while protonation at one terminal ring nitrogen would imply a more complex spectrum due to the asimmetry of the molecule. The ^{13}C-NMR spectrum of **5c**, not reported here, in the same solvent and at the same pH, shows 10 signals attribuitable to 10 magnetically equivalent carbon atoms, pointing out further evidence for the simmetry of the mono-protonated molecule. Di-protonation at two terminal ring nitrogens, which would also provide a spectrum with only 10 carbons, can be ruled out by the characteristic 4' carbon downfield shift (+ 4.6 ppm) consistent with those reported [12] for pyridine ring nitrogen protonation (+ 3.5 ppm) or methylation (+7.1 ppm).
The above considerations provide evidence in favour of the first protonation occurring at the central pyridine nitrogen.

EXPERIMENTAL

Melting points are uncorrected. IR Spectra (KBr discs) were recorded on a Perkin-Elmer Model 684 spectrophotometer. The 200 MHz ^{1}H NMR spectra were recorded at 25°C in $CDCl_3$ (20 mg/ml) on a Bruker AC 200 spectrometer with a 4s pulse repetition, a 2000 Hz spectral width, 16K data points, and 250 scan accumulation. The ^{1}H decoupled 500 MHz ^{13}C NMR spectra were recorded on the same samples with a 1.6 s pulse repetition, a 10000 Hz spectral width, 32K data points, and a 30000 scan accumulation. Mass spectra were recorded by direct insertion probe into the ion source of a Q MD 1000 quadrupole mass spectrometer (Fisons) using the Lab-Base acquisition and processing software. Mass spectra were acquired with a 3 scan time and 3 s interscan delay, using 70 eV ionization energy. The source temperature was maintained at 200°C.

pKa Determinations

Mixtures of methylcyclohexane/3-methylpentane (9/1, v/v) and of water/acetonitrile (9/1 to 8/2, v/v) were used as solvents. The absorption spectra were recorded by a Perkin Elmer Lambda 16 spectrophotometer.
The fluorescence spectra, corrected for the instrumental response, were recorded by a Spex Fluorolog-2 FL 112 spectrofluorimeter which utilizes Rhodamine B as a quantum counter, controlled by the Spex DM3000f spectroscopy computer. The acid-base equilibria were studied in aqueous solutions (containing 10% or 20% MeCN for solubility requirements) at room temperature by both spectrophotometry and fluorimetry. Britton buffer solutions were used from pH 10 to pH 2, at costant ionic strength (μ=0.02). The pH values of the solutions were

controlled by an Orion digital pH-meter SA-520 equipped with an Orion 9103 SC semimicro electrode standardized by aqueous buffers. $HClO_4$ solutions were used in the higher acidity range. No corrections for the activity coefficients were applied.

Solutions were freshly prepared and kept in the dark to avoid *trans-cis* photoisomerization. For the fluorimetric measurements, the solutions (deoxygenated by bubbling pure nitrogen) were excited at an isosbestic point between the absorption spectra of neutral and protonated forms.

Syntheses

Divinyl pyridines **5** were synthesized according to methods A and B (Scheme 1) in the absence of light.

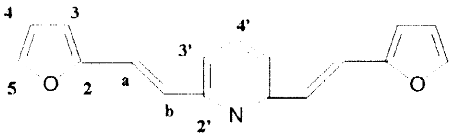
Method A:

A solution of 2,6 dimethylpyridine (1.5 mmoles) and heteroaromatic aldehyde (4.5 mmoles) were refluxed in 3 ml of acetic anhydride for 1 to 5 hours. The mixture was purified in a silica gel cromatographic column, using cyclohexane/ethyl acetate as eluent.

Method B:

A solution of 2,6-pyridindicarboxaldehyde (1.5 mmoles) and 2-picoline (4.5 mmoles) was refluxed in 3 ml acetic anhydride for 3 hours. The composition of the mixture was examined by TLC. To obtain the pure product it was necessary to purify the mixture in a cromatographic column, using cyclohexane/ethyl acetate as eluent.

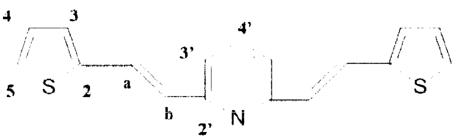
2,6-di-[2-(furan-2-yl) vinyl]pyridine (5a):



Yield 45% (after chromatographic purification), Method A, time 3 hrs., yellow needles, R_f 0.63 (cyclohexane/ethyl acetate, 3/1, v/v), m.p. 104-105 °C. **MS, m/z (%):** 263 (M^+, 100), 246 (10), 235 (18), 234 (65), 220 (20), 204 (12), 182 (20), 180 (9), 167 (8), 155 (10), 154 (90).

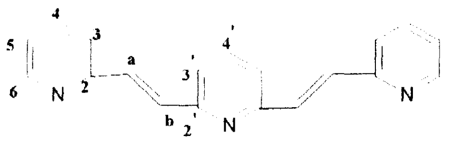
^{1}H NMR ($CDCl_3$) δ (ppm): 7.58 H_b (2H, d, J= 15.77 Hz), 7.06 H_a (2H, d, J=15.77 Hz), 7.58 $H_{4'}$ (1H, t, J=7.85 Hz), 7.12 $H_{3'}$ (2H, d, J=7.56 Hz), 7.44 H_5 (2H, m.), 6.47 H_4 (2H, dd, J=3.33, J=5.1 Hz), 6.44 H_3, (2H, d, J=3.33 Hz). **^{13}C NMR ($CDCl_3$) δ (ppm):** 154.6 (C-2'), 153.0 (C-2), 142.7 (C-5), 136.9 (C-4'), 126.1 (C-a), 120.9 (C-3'), 120.3 (C-b), 111.8 (C-4), 110.5 (C-3).

2,6-di-[2-(thien-2-yl) vinyl]pyridine (5b):



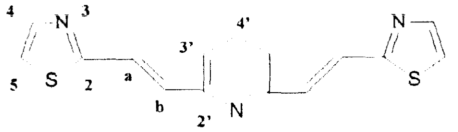
Yield 30% (after chromatographic purification), Method A, time 5hrs., yellow needles, R_f 0.75 (cyclohexane/ethyl acetate, 5/1, v/v), m.p. 165-7 °C. **MS, m/z (%)**: 295 (M^+, 12), 236 (30), 167 (100), 154 (90), 141 (18), 97 (25), 58 (12), 45 (38), 39 (20). **^{1}H NMR, ($CDCl_3$) δ (ppm)**: 7.85 (2 ethylenic H, d, J= 15,85 Hz), 6.98 (2 ethylenic H, d, J= 15.85 Hz), 6.9-7.3 (9H, multiplets). **^{13}C NMR ($CDCl_3$) δ (ppm)**: 154.9 (C-2'), 142.3 (C-2), 136.9 (C-4'), 127.7, 127.6, 127.5, 125.8, 125.4, 120.4 (C-3, C-4, C-5, C-3', C-a, C-b not assigned).

2,6-di-[2-(pyridin-2-yl)vinyl]pyridine (5c):



Yield 15% (after chromatographic purification), Method B, time 3 hrs, pale yellow microcrystals, R_f 0.55 (cyclohexane/ethyl acetate, 3/1, v/v), m.p. 152-4°C. **MS, m/z (%)**: 285 (M^+ 13), 284 (20), 228 (23), 227 (100), 211 (67), 210 (52), 209 (85), 199 (14), 181 (20), 136 (18), 108 (20), 93 (46). **^{1}H NMR, ($CDCl_3$) δ (ppm)**: 8.65 H_6 (2 H, m, J=3.2 Hz), 7.87 H_b (2H, d, J= 15.7 Hz), 7.69 H_a (2H, d, J= 15.7 Hz), 7.71 H_5 (2H, t, J=7.6 Hz), 7.67 $H_{4'}$ (1H, t, J=7.7 Hz), 7.52 H_3 (2H, d, J=7.83 Hz), 7.33 $H_{3'}$ (2H, d, 7.7 Hz) 7.19 H_4 (2H, ddd, J=1.1, J=3.2, J=7.6 Hz). **^{13}C NMR ($CDCl_3$) δ (ppm)**: 155.1 (C-2'), 154.7 (C-2), 149.6 (C-6), 137.2 (C-4'), 136.6 (C-4), 132.0 e 131.9 (C-a and C-b), 122.9 (C-5), 122.6 and 122.3 (C-3 and C- 3').

2,6-di-[2-(thiazol-2-yl) vinyl]pyridine (5d):



Yield 15% (after chromatographic purification), method A (molar ratio 1:4), time 1hr, yellow microcrystals, R_f 0.16 (cyclohexane/ethyl acetate, 2/1, v/v), m.p 198-200°C. **MS, m/z (%)**: 297 (M^+, 100), 264 (42), 238 (65), 226 (90), 212 (35), 199 (18), 168 (60), 155 (30), 142 (15), 86 (18), 58(5). **^{1}H NMR, ($CDCl_3$) δ (ppm)**: 8.169 H_a (2H, d, J=16 Hz), 8.067 H_4 (2H, d, J=3), 7.892 $H_{4'}$ (1H, t, J=7.5 Hz), 7.71 H_b (2H, d, J=16 Hz), 7.535 H_5 (2H, d, J=3 Hz), 7.51 $H_{3'}$ (2H, d, J=7.5 Hz). **^{13}C NMR ($CDCl_3$) δ (ppm)**: 166.2 (C-2), 154 (C-2'), 143.9 (C-5), 137.4 (C-4'), 132.7 (C-4), 125.4 (C-b), 122.6 (C-a), 119.3 (C-3').

2,6-distyryl pyridine (5e): m.p. 162-4 °C (lit. 165-6.5 °C) [13].

MS, m/z (%) : 284 (M^+ + 1, 6), 283 (M^+, 32), 282 (M^+-1, 86), 204 (40), 191 (30), 178 (10), 152 (20), 128 (15), 115 (15), 102 (40), 91 (42), 78 (47), 77 (100), 65 (12), 63 (22), 51 (50), 39 (20).

ACKNOWLEDGMENTS

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